Perturbing in place: Systems for in situ and cell-type specific single-cell genetic screens

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Abstract

Glioblastoma multiforme (GBM) is a condition that impacts 3 in 100,000 persons, accounting for more than 100,000 cases annually in the United States alone. The standard of treatment for a GBM entails a maximum amount of surgical resection, radiation, and adjuvant temozolomide (TMZ). Unfortunately, TMZ is ultimately ineffective since it has been associated with both inherent and acquired drug resistance.[1] In this study I applied various cloning methods to construct an *in-situ* cell-type specific gene editing tool by placing two adjacent sgRNA components into an avian retroviral vector. By modeling how genetic background alters evolution in response to drug exposures, this tool has the potential to identify opportunities for improved GBM therapies.

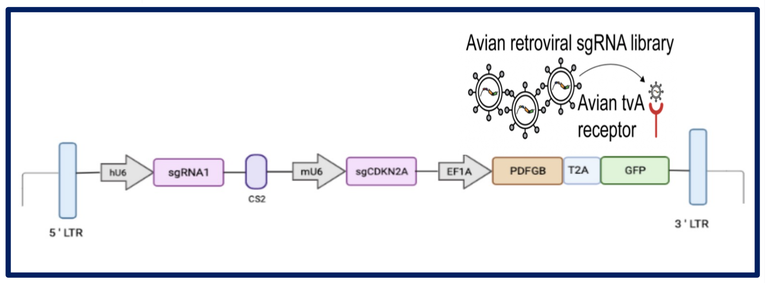


Figure 1. Final result after constructing avian retroviral vector.

Introduction

GBM is a malignant brain tumor that develops from a specific type of brain cell called an astrocyte. GBMs are often very aggressive and spread to surrounding tissues. Only 3.3% and 1.2% of patients survive the illness after two and three years, respectively. [2] Some of the most common symptoms are persistent headaches, seizures, memory loss, changes in mood and personality, and speech difficulty. [3] Barely 6.8% of glioblastoma patients survive after five years, and their average survival time is only eight months, according to estimates.[4] The complexity of the tumor itself is one of the causes of GBM's resistance to therapeutic intervention.

While GBM has been shown to have a ubiquitous over-activation of RTK signaling, GBM patients display low response rates to RTK targeted therapy. Adaptive activation of pathways that rescue RTK signaling is suspected to be one of the mechanisms by which tumors evade therapy. Hence, there is a need to map how tumors shift expression programs in response to various drug exposures. Patchwork provides a platform to fulfill this need via *in situ* cell-type specific CRISPR screens.

As proof that the final Patchwork plasmid tool effectively delivers the expected genetic perturbations, we inserted either single guides HPRT1 or NTC in place of the sgRNA component. HPRT1 confers resistance to chemotherapy 6-TG and NTC is a non-targeting control. We transfected DF1 chicken cells via Lipofectamine and planned to use the virus produced by these cells to transduce DF1 and T98G dCas9 KRAB +/- tvA mCherry human GBM cells. Assuming the Patchwork plasmid is effective, only cells with tvA receptor (i.e. DF1 and T98G dCas9 KRAB + tvA mCherry) will receive the intended genetic perturbations. Consequently, T98G dCas9 KRAB + tvA mCherry cells transduced with Patchwork (HPRT) will be resistant to 6-TG treatment whereas T98G dCas9 KRAB cells will not.

Methods

Gibson Ligation

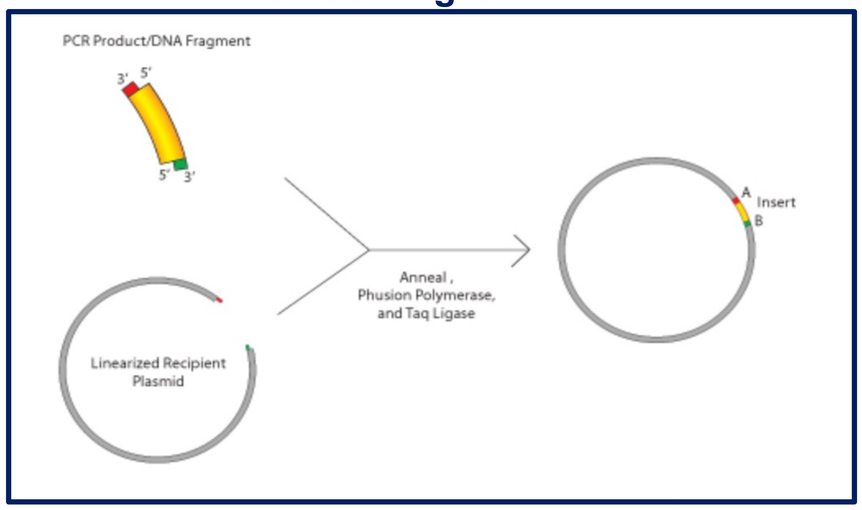


Figure 2. We ligated single guide RNAs, namely HPRT1, and NTC, and a mU6 sgCDKN2A gBlock into the pDonor vector via Gibson Assembly.

Clonase

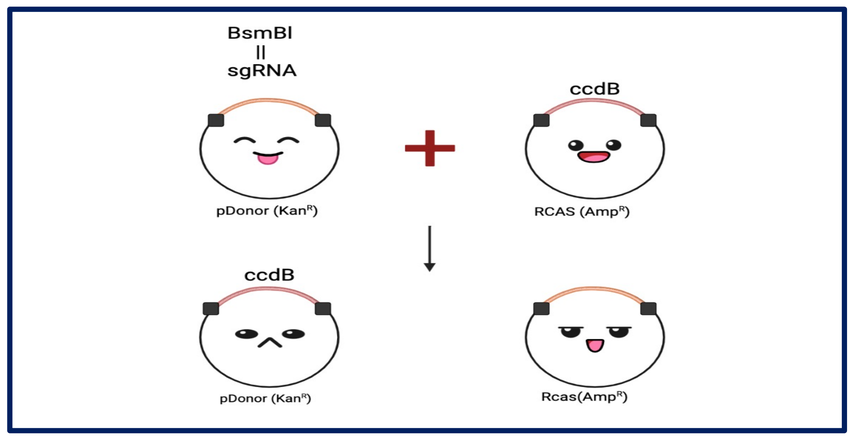


Figure 3. During the Clonase reaction, the ccdB is exchanged with the Patchwork gene editing constructs. Since the ccdB protein inhibits the growth of ccdB sensitive E. coli strains and pDonor vector does not contain ampicillin resistance, most colonies should contain RCAS vector with the HPRTI & NTC guide when grown on an ampicillin plate.

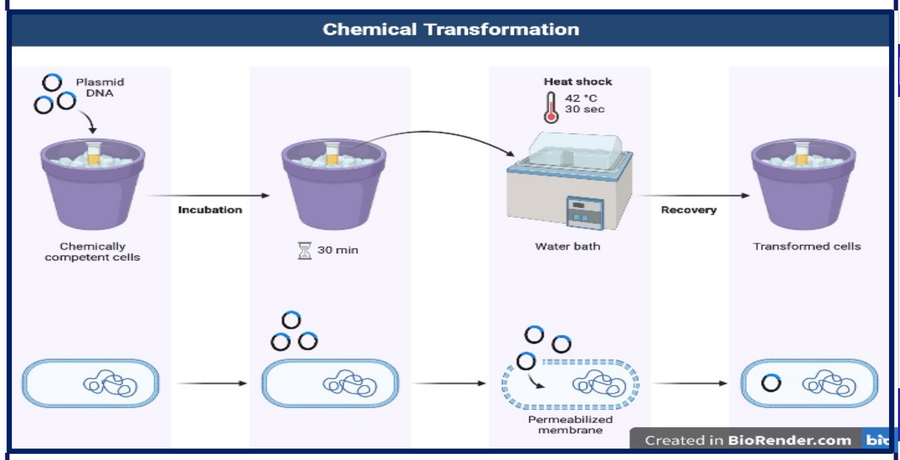
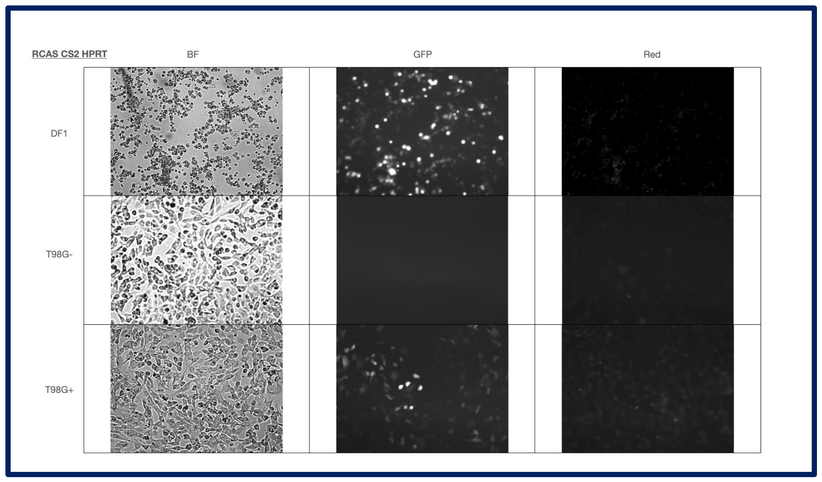
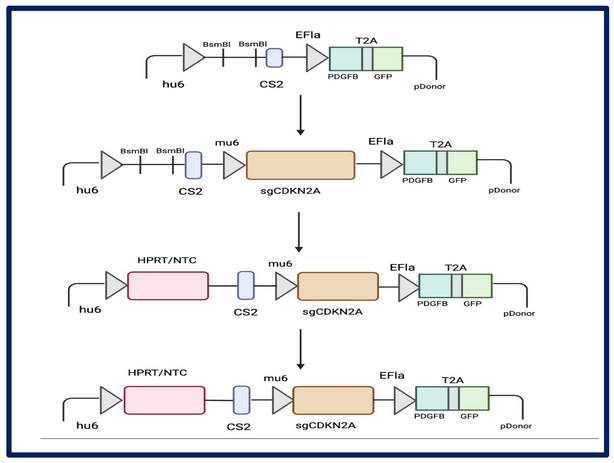


Figure 4. We use transformation to replicate the plasmids as well as confirm that the Clonase & Gibson Ligation reactions were successful.

Results

Transduction & step-by-step construction of Patchwork





Future Work

- Transduction of T98G dCas9 KRAB, T98G dCas9 KRAB tvA mCherry and DF1 cells
- In vitro experiments with mouse embryonic stem cells and embryoid bodies to modulate neural cell lineages.
- In vivo experiments with Nestin-tvA and GFAP-tvA mice: induce tumors, knock out specific genes, introduce chemical perturbations (drug treatments) and sequence.
- Applications in precision medicine

References

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